JOURNAL
OF THE
WASHINGTON ACADEMY OF SCIENCES

Vol. 44 March 1954 No. 3

BIOCHEMISTRY.—*The reciprocal effects between calcium and phosphate ions upon the growth, composition, and structure of castor bean,* Ricinus communis L.[1] Frank D. Venning, Swingle Plant Research Laboratory, University of Miami. (Communicated by J. R. Swallen.)

The present investigation was undertaken to determine the effects of calcium and phosphorus deficiency on the growth and development of the main axis of castor bean, *Ricinus communis* L., and to determine the possible influence of the interaction between calcium and phosphorus ions on its growth and development. Comparisons between the composition and structure of plants grown with and without calcium, with and without phosphorus, and with and without both calcium and phosphorus are given, and possible cause and effect relationships are pointed out. New data are presented concerning growth phenomena as expressed by the axis of castor bean, and correlations between such growth phenomena and the chemical composition of the tissues involved are offered.

MATERIALS, METHODS, AND PROCEDURES

Methods of Culture of the Experimental Plants

Certified seeds of *Ricinus communis* L. var. *cambodgensis* J. B. S. Norton were germinated in greenhouse flats of sand, watered with tap water, and 12 days after germination were transplanted to four hydroponic culture series: (1) Controls (2) minus calcium, (3) minus phosphorus, and (4) minus both calcium and phosphorus.

For each culture series 10 2-gallon glazed stoneware jars were employed, filled with No. 10 mesh washed quartz gravel for root anchorage and aerated by a regulated flow of washed compressed air introduced into the bottom of each jar. Three seedlings were planted in each jar, providing 30 plants in each series. A modified Knop's solution was used as a nutrient medium in all four series (Table 1), and the jars were kept filled with the solution to a level just beneath the surface of the gravel. In addition, micronutrients of boron, zinc, manganese, copper, and iron were supplied to all series of plants. The original pH of the solutions is noted in Table 1.

At the time of transplanting the seedlings to the cultures the nutrient solutions were diluted to supply a concentration of 0.01 per cent of total solutes; beginning a week later the level of total solutes in all series was gradually increased by adding small amounts of nutrient solution containing 0.1 percent of total solutes from time to time as required by the plants, until at the end of a 4-week period a concentration of 0.1 percent of total solutes was achieved in the nutrient solutions of all four series of plants. The series were thereafter grown at 0.1 percent of total solutes for the remainder of the experiment; there were no observable adverse effects on the plants of a nature which would suggest that the osmotic pressure of the solution was unfavorable to the growth of castor bean.

Weekly checks of the pH of the nutrient solutions in the cultures were made, and it was noted that the pH in the control series and to some extent in the series lacking both calcium and phosphorus tended to rise above 7.0, presumably because of unequal uptake of ions. The cultures were artificially maintained in a pH range between 6.5 and 7.0 by the addition of small amounts of HCl when indicated, as some

[1] This study was undertaken as a part of a course at the State University of Iowa, and the author is indebted to Dr. Walter F. Loehwing and Dr. Robert L. Hulbary of that institution for their personal interest and suggestions. The study was completed in its present form at the Swingle Plant Research Laboratory of the University of Miami.

foliar chlorosis develops in castor bean when the pH rises above 7.2. It was determined that the chlorosis was caused by insufficient iron uptake in the alkaline medium, which was quickly overcome when the pH was maintained slightly below 7.0.

TABLE 1.—COMPOSITION OF THE NUTRIENT SOLUTIONS

1. CONTROL (pH 5.35)
   - 2g KNO_3 — 2g KH_2PO_4
   - 2g $MgSO_4$ — 8g $Ca(NO_3)_2$
   - Distilled water to make 14 liters.
2. MINUS CALCIUM (pH 5.4)
   - 2g KNO_3 — 2g KH_2PO_4
   - 2g $MgSO_4$ — 4g $Mg(NO_3)_2$
   - 4g $NaNO_3$ — Distilled water to 14 liters.
3. MINUS PHOSPHORUS (pH 5.9)
   - 2g KNO_3 — 1g KH_2SO_4
   - 1g KCl — 2g $MgSO_4$
   - 8g $Ca(NO_3)_2$ — Distilled water to 14 liters.
4. MINUS CALCIUM AND MINUS PHOSPHORUS (pH 6.1)
   - 2g KNO_3 — 4g $Mg(NO_3)_2$
   - 2g $MgSO_4$ — 1g KCl
   - 1g KH_2SO_4 — 4g $NaNO_3$
   - Distilled water to 14 liters.

All four series of plants were grown simultaneously in the same greenhouse under comparable conditions of light, humidity, and temperature. Since the castor bean is of tropical origin and these investigations were begun in the north at latitude 42° in the month of January, the photoperiod was extended to 12 hours per day by means of 16 20-watt fluorescent lamps hung 6 feet above the greenhouse bench. This extension of the photoperiod allowed the plants to develop under conditions more nearly approximating those which would be considered "normal" for castor bean.

At the beginning of the experiment the day temperatures were maintained at 75°F., and night temperatures at 65°F., the day temperature coinciding with the period of illumination. Fifty-two days after their introduction into the culture media small flower buds were noted on several plants in all four series, and the temperatures were raised to 75°F. night—85°F. day, and maintained there during the balance of the experiment, a total of 133 days.

Sampling: Anatomical Data

Growth within any particular series was relatively uniform for all plants in that series. Because of this uniformity the plants were not sampled at random but were sampled selectively: The three plants having the longest, shortest, and the most nearly medium-sized axis were selected from each series, and the anatomical and morphological data were based on their development. Within the four series the dates of sampling were staggered to give more nearly comparable results, and were as indicated: Controls: after 113, 118, and 122 days in the cultures; Minus calcium: after 116, 120, and 123 days; Minus phosphorus: after 116, 121, and 124 days; and the series Minus calcium and minus phosphorus after 117, 122, and 125 days in the nutrient cultures. At this time all elongation had ceased in the lower portions of the axis, and secondary radial growth was relatively slow. The seven basalmost internodes were selected for study because they were all differentiated at approximately the same time in all four series, and the eighth node had been differentiated before the inception of any floral primordia. The tissue of these lower internodes was thus the most comparable, physiologically, between the series.

The tissues composing the axis of castor bean are arranged as a series of fairly regular concentric cylinders, with the exception of a small region at each node where the leaf trace leaves the stele, and which was ignored for the purposes of this study. Several transverse and longitudinal free-hand sections were made near the center of each internode, stained in a 1:10,000 solution of neutral red in distilled water, and mounted in glycerin for microscopic study. Eight transverse measurements were made of the width of each of the major tissue regions within each internode of each plant. As successive nodes are not at an exact right angle to the sides of the internode, the length of a particular internode was determined as the average of its longest and shortest length. From averages of the data so obtained it was possible to determine the transverse-section area and actual volume of all major tissues within a particular internode.

The sections were also used for volumetric studies of the pith cells. Tangential, radial, and longitudinal measurements were made of 50 pith cells from each internode. Those pith cells nearest the center of the internode are the largest in all three dimensions. They gradually decrease in size as one moves toward the periphery of the pith; the smallest cells bordering on the medullary sheath are from one-third to one-fourth as large in any one dimension as those nearest the center (the immediate center of the more mature internodes is hollow). In order to secure cell measurements which would afford a relatively

average picture of the development of the pith within a particular internode, and because of the general progression of size in the cells as described, these cells were not sampled at random; all of the pith cells which lay along two vectors which intersected at right angles in the center of the internode were measured along their greatest radial and tangential axes. There were usually between 12 and 13 pith cells along a vector from the medullary sheath to the hollow center of the internode. The 50 longitudinal measurements were made in a similar manner, i.e., the same number of cells was measured along vectors extending at right angles from the medullary sheath to the center of the internode.

Sampling: Histochemical Data

Standard histochemical testing procedures as described by Chamberlain (1932), Johansen (1940), and Foster (1949) were employed to determine the presence and extent of a number of substances in the seven basal internodes. It was usually possible to determine in which tissue a particular substance was present, and also possible, within the limits of sensitivity of the tests, to determine relative quantities of a particular substance. Five plants from each series were employed for this purpose, and the tests performed on fresh freehand sections usually made from the center of the internode, but frequently from the upper and lower portions of each internode as well. A part of these determinations were made during the period of anatomical sampling, and the remainder were performed within the subsequent week; the plants had thus been growing in the cultural solutions for a period of 126 to 133 days.

OBSERVATIONS

General Growth and Development of the Four Series of Plants

During the period of vegetative growth the axis of castor bean is monopodial. One leaf is produced at each node; the axillary buds remain dormant and the axis is unbranched. With the advent of flowering the habit becomes sympodial; in addition to the leaf a terminal inflorescence is produced at each node, and the axillary bud develops the successive internode. The stem is thus of indeterminate growth, and the plant is a perennial under favorable environmental conditions.

During the entire vegetative phase of growth of the plants studied in this experiment, differentiation of nodes and leaves took place at the same rate in all four series; i.e., successive nodes and leaves became visible on all plants after the same time interval had elapsed. During this phase the lower internodes of all plants were approximately of the same diameters and lengths, but sensitivity to differences in nutrient supply was soon apparent in the leaves. The control series, with a complete nutrient supply, had foliage with the largest leaf area, deep green in color. The leaves of plants deficient in calcium had an area approximately two-thirds as great as those of the control, somewhat wrinkled in texture, and yellow-green in color. Plants lacking phosphorus had a leaf area about three-fourths as great as the control, and developed the deep blue-green hue frequently associated with phosphorus deficiency. The plants deficient in both calcium and phosphorus more closely resembled the control than did either of the series deficient in calcium or phosphorus alone. The leaves were of comparable color and size to those of the controls.

The uptake of nutrient solution during the vegetative phase of development paralleled general size and leaf area of the plants. It was greatest in the controls, next largest in the series minus both calcium and phosphorus, and least in the series minus calcium.

Fifty-two days after their transfer to the nutrient solutions, inflorescences were noted on several plants in each of the four series, and within a few days the first inflorescence had appeared on almost all of the plants. The first inflorescences were differentiated at either the eighth or ninth nodes on all plants.

After entering the flowering phase, profound changes in development took place between the four series of plants. The control plants continued to develop vigorously, and fruits were set and matured from the lower flower spikes while new foliage and inflorescences were continuously differentiated at each successive node. The leaves produced at the flowering nodes were somewhat larger than those from vegetative nodes; the blade width averaging slightly over 30 cm. Nutrient uptake in this series gradually increased throughout the course of the experiment. At the time of sampling, the roots were noted to be clean and firm, much-branched, and in active growth.

In the series minus calcium, flowering brought about an abrupt cessation of elongation in the axis. For a time nodes and internodes continued to be differentiated at the same rate as those of the control, but subsequent elongation of the internodes was greatly inhibited, both in the newly-formed "floral" internodes and in the younger internodes which had been differentiated prior to the appearance of the first inflorescence. Increase in stem diameter was also retarded, but not to as great a degree. The inflorescences produced were rudimentary, and became increasingly so at each successive node. A few of the male flowers of the first inflorescences produced pollen, but for the most part the male flowers were sterile; and no fertile female flowers were produced in any of the inflorescences. Foliage differentiated at flowering nodes was much reduced in size; most leaves had a blade under five centimeters wide. This foliage was pale yellow or almost white, brown-spotted, curled or wrinkled, and ephemeral. The leaves on the vegetative portions of the axis were gradually abscised, and as defoliation increased, the rate of growth of the apical portions of the axis fell behind that of the control and finally ceased altogether. Uptake of nutrient solution decreased sharply after the initiation of flowering, and continued to decrease during this phase of development. Many of the plants in this series remained alive until the termination of the experiment, but all had ceased any visible development. At the time of sampling the roots were noted to be soft, relatively few-branched, and considerable sloughing of root tissue was evident.

In the series deficient in phosphorus, the first inflorescence bore fertile male and female flowers, and young fruits were set. As development of the first fruits began, the lower leaves developed dark brown spots, became flaccid, and were promptly abscised, so that the "vegetative" portions of the axes of these plants were almost completely defoliated within a week. Elongation of the younger "vegetative" internodes and newly-formed "floral" internodes was inhibited, but not to so great a degree as in the plants lacking calcium, and their lateral expansion was only slightly inhibited. The foliage produced at the flowering nodes was smaller than that formed previously, with a blade width between 10 and 15 cm. The rate of differentiation of the subsequent internodes was slowed, and if young fruits were forming on the first inflorescence, the female flowers were abscised from all subsequent inflorescences. On the lower inflorescence, if several fruits were developing, all but one were abscised; and this remaining fruit required a longer period to mature than did the earliest fruits of the control. At maturity the seeds were found to be undersized, imperfectly formed, and contained almost no endosperm. The embryo was rudimentary, and the seeds were nonviable. Plants of this series which matured a fruit as described died when the capsule matured and dehisced. A few plants in this series did not enter the floral phase of development; these continued vegetative growth for the duration of the experiment. As new leaves were differentiated the older foliage underwent the discoloration and abscission as described for the plants which flowered, but the abrupt loss of foliage did not take place; the new leaves were larger, attaining a width of 20 cm. The uptake of nutrient solution decreased with defoliation, and remained at a relatively low level thereafter. The roots of these plants were not so extensive as those of the control, but appeared to be healthy and in slow growth at the time of sampling.

The series lacking both calcium and phosphorus reacted to flowering in a manner similar to the plants grown without phosphorus, but with several important differences. At first they showed none of the outward effects seen in the series deficient in calcium. The lower inflorescences set fruits, and discoloration and abscission of the lower leaves followed, but defoliation took place more slowly in this series than in that lacking only phosphorus. Subsequent inflorescences did not set fruits, but the elongation of the internodes was inhibited less in this series of plants. Two or three fruits were ultimately matured on the lower inflorescence; they were slow to ripen, undersized, with small seeds, but endosperm was present, the embryo appeared normal though reduced in size, and several were viable. During maturation of the fruits the rate of differentiation of the upper nodes and internodes was retarded below the control, and the new foliage produced during the maturation phase resembled that of the plants in the series minus calcium. The leaves were about 10 cm broad, somewhat wrinkled and chlorotic, but never as much so as those of the plants lacking calcium alone, and they were fairly persistent. Plants in this series did not die at the maturation of the fruits, but subsequent differentiation of nodes, as well as stem elonga-

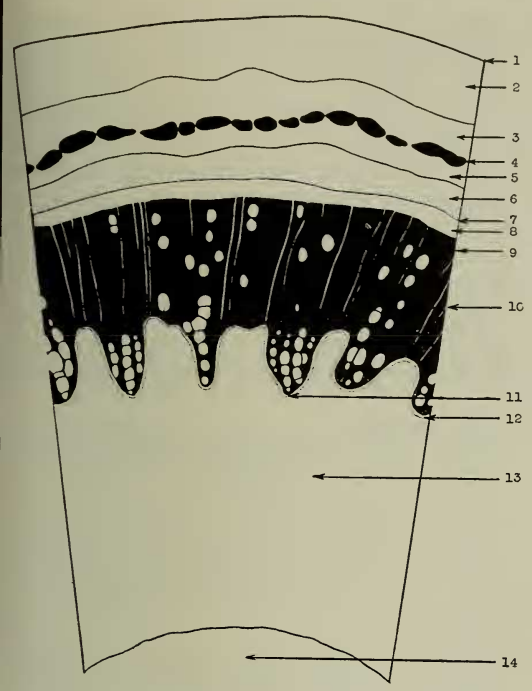

FIG. 1.—Diagrammatic transverse-section area of a segment of the axis of castor bean, indicating the general tissue regions in the axis and their arrangement: 1. *Epidermis*, composed of a single layer of small brick-shaped cells which bear on their outer face a cuticular layer. 2. *Outer cortex*, or *hypodermis*, is made up of relatively small cells of isodiametric shape, for the most part collenchymatous, containing abundant chlorophyll. 3. The *inner cortex* is parenchymatous, the cells highly vacuolate, more or less isodiametric, chlorophyll-bearing, and of larger individual volumes than the cells of the hypodermis. 4. *Sclericycle* is several cells deep, an interrupted layer of mechanical tissue with greatly thickened cell walls, and whose protoplasts are dead at maturity of the elements. Ontogenetically it is probably a part of the primary phloem. 5, 6. The *phloem* contains two distinct regions: The outer, older, primary phloem (5) contains a high proportion of phloem parenchyma, quite large amounts of chlorophyll, and is probably no longer active as conductive tissue. It is for the most part of primary derivation. The inner, younger, secondary phloem (6) is principally composed of phloem parenchyma, sieve tubes, and companion cells; is derived from the vascular cambium. 7. The *vascular cambium* is continuous around the stem, and appears as a thin cell layer one cell in thickness. 8. *Secondary xylem parenchyma* is the tissue lying to the inside of the vascular cambium, and which has not as yet undergone differentiation into one of the more complex xylem elements. 9. *Xylem* is represented by the large black area in the diagram. The bulk of the secondary xylem is composed of small elongated cells with pitted lignified walls, tapering ends, and living protoplasts. These mechanical and storage elements, the xylem prosenchyma, are derived from the subsequent differentiation of cells which arise from the activity of the vascular cambium. 10. *Pith rays* are usually only one or two cells wide, may have a thin deposition of lignin in their walls, and usually extend from the medullary sheath across the vascular cylinder to the cortex. They are represented in the diagram by white lines in the xylem. 11. The first formed conducting elements and protoxylem parenchyma, both of which are primary in origin, lie to the inside of the secondary xylem cylinder as a series of small vertical ridges which project into the pith, the so-called *protoxylem points*. The largest number of vessels (represented by white circles) are found in these protoxylem points. 12. *Medullary sheath:* The outermost layers of the pith, one to three cells deep and abutting on the inner face of the xylem cylinder. They differ from the remainder of the pith cells in their smaller transverse diameter, greater length, and intercellular spaces much reduced or lacking. 13. The *pith* is the innermost tissue. The cells are parenchymatous, isodiametric in transverse view, and highly vacuolate. 14. Hollow in the center of the axis of the more mature internodes.

tion, was greatly retarded. Uptake of nutrient solution became more and more retarded as the leaves were abscised, and, while the uptake was greater than in either of the series lacking only one macronutrient, was far less than the control plants. Root development at the time of sampling was comparable to the plants grown without phosphorus, and there were no evidences of deterioration of root tissue.

Structure and Development of the Basal Internodes

The tissue of the internodes of the main axis of castor bean is at first solid, but unequal rates of differentiation and expansion of the inner and outer tissue regions result in a hollow area extending down the center of each internode as it matures. All axial tissues are differentiated as a series of concentric cylinders of fairly regular outline as shown in Fig. 1. At the time of sampling, both primary and secondary tissues were present in the axes of all plants. As is seen in Fig. 1, the axis of castor bean is of regular and conservative structure, free from growth anomalies. Under the conditions of extreme deficiencies of calcium and phosphorus in this experiment, no changes in anatomical "patterns" or tissue arrangement occurred. The tissues as described in Figure 1 were developed in all series of plants; the response to variations in nutrients was expressed by differences in quantities of tissues and their proportions to one another. Such differences in quantities reflected differences between the activities of the meristems, both apical and vascular, and also reflected differences in the amount of secondary differ-

entiation into specialized tissues from cells originated by these meristems.

As the quantity of primary vascular tissue is small, the bulk of the vascular tissue is secondary and the area of xylem and phloem affords an index of cambial activity within the axis. These data are presented for all series of plants in Tables 2 to 5. For purposes of measurement, the medullary sheath was included with the pith; xylem was measured from the medullary sheath to the cambium; phloem from the cambium to the outside of the sclericycle (as these cells are probably primary phloem derivatives); and the cortex included all tissue between the sclericycle and epidermis.

As shown in Tables 2 to 5, the larger areas of secondary tissues are found in the first basal internode, and the proportions of vascular to nonvascular tissue decreased in each successive internode. The diameter of the pith is successively increased; whereas the transverse-section area of cortical tissues is approximately constant.

In the control plants there is a marked tendency for each successive internode to elongate to a greater degree than the previous internode; this trend continues until "floral" internodes are differentiated; from the first node bearing an inflorescence upward the internodes are of more or less equal length.

In the series lacking calcium, cambial activity was greatly reduced, particularly in the upper internodes. In the fifth and sixth internodes, most of the secondary xylem indicated in Table 3 was xylem parenchyma, cells of cambial origin which had not undergone any differentiation into functional xylem elements. There was little secondary xylem in the seventh internode. On the other hand, the primary xylem was well differentiated into conducting elements in these upper internodes. A small amount of phloem in the upper internodes appeared to be derived from the vascular cambium. The length of the internodes in this series decreased progressively upward.

The first three internodes of the plants grown without phosphorus showed similar developmental patterns to those of the control, with a progressive increase of length of the internode. The length of the remaining vegetative internodes tended to become constant, with decreased length occurring in the "floral" internodes. The transverse-section area of vascular tissue progressively decreased, but the elements were for the most part well differentiated into conducting and mechanical tissue.

The tissues of plants grown without both calcium and phosphorus more nearly resembled the controls than did either of the series grown without calcium or phosphorus alone. Progressive increases in internodal length were similar for the first five internodes; thereafter the internodes became progressively shorter. Secondary vascular tissues were well differentiated in all seven internodes.

The average volumes of the various tissues in the first seven internodes were computed from

TABLE 2.—TISSUE AREAS AND LENGTHS IN THE SEVEN BASAL INTERNODES OF THE CONTROL SERIES[1]

	AREA OF: (Areas in mm²)				LENGTH OF INTERNODE
	Pith	Xylem	Phloem	Cortex	
					mm.
INTERNODE 1					
Plant 1	12.26	43.10	13.12	8.09	17.5
Plant 2	15.49	53.18	13.97	8.86	16.5
Plant 3	10.24	58.05	15.31	9.69	22.5
Average	12.66	51.44	14.13	8.88	18.83
INTERNODE 2					
Plant 1	13.91	41.10	13.39	7.60	14.5
Plant 2	18.16	49.68	14.25	7.55	24.0
Plant 3	16.51	51.81	13.91	8.38	17.5
Average	16.19	47.52	13.85	7.84	18.67
INTERNODE 3					
Plant 1	17.17	36.42	10.82	8.98	17.0
Plant 2	20.20	43.64	13.04	7.43	19.5
Plant 3	15.87	47.88	13.23	7.72	25.0
Average	17.75	42.65	12.36	8.04	20.5
INTERNODE 4					
Plant 1	30.74	26.78	11.21	8.43	24.0
Plant 2	36.21	36.71	13.18	8.86	25.3
Plant 3	24.25	42.62	12.81	7.21	29.0
Average	30.40	35.37	12.40	8.17	26.1
INTERNODE 5					
Plant 1	37.61	21.47	10.57	8.51	35.0
Plant 2	41.69	31.03	11.13	9.59	39.0
Plant 3	32.13	37.25	11.95	8.11	38.0
Average	37.14	28.92	11.22	8.73	37.33
INTERNODE 6					
Plant 1	39.70	21.80	10.75	9.29	32.0
Plant 2	38.32	22.85	10.02	7.85	40.0
Plant 3	38.44	31.69	11.07	8.78	47.0
Average	38.82	25.45	10.61	8.64	39.66
INTERNODE 7					
Plant 1	45.81	17.16	10.50	8.98	40.0
Plant 2	45.86	20.33	9.73	8.35	38.5
Plant 3	38.90	25.26	10.32	7.66	56.0
Average	43.52	20.92	10.18	8.33	44.83

[1] Internode 1 is basalmost.

the data of Tables 2 to 5, and are presented numerically in Table 6, and diagramatically in Figs. 2 to 5.

The length of any axis segment, or internode, is primarily determined by the number of tiers of cells within the unit, and the degree of elongation which they have undergone; whereas the transverse-section areas of primary tissues, such as the pith and cortex, are determined by the width of the apical meristem, and the subsequent degree of intercalary growth and expansion of the cells. In all four experimental series, the size of the individual secondary vascular elements was approximately the same, but the size of primary elements, such as the cortex and pith cells, was dissimilar. The rate and degree of activity of the apical meristem is directly reflected in the number of nodes produced, and the actual number of cells in the primary tissues of the internodes. As the pith had the largest overall volume of any tissue in the axis, and is the first tissue to terminate meristematic activity and undergo vacuolation during the ontogeny of the internode, its cellular makeup was selected as an index of the activity of the apical meristem at the time the lower internodes were differen-

TABLE 3.—TISSUE AREAS AND LENGTHS IN THE SEVEN BASAL INTERNODES OF THE SERIES MINUS CALCIUM[1]

	AREA OF: (Areas in mm²)				LENGTH OF INTERNODE
	Pith	Xylem	Phloem	Cortex	
					mm.
INTERNODE 1					
Plant 1	8.69	6.72	4.03	4.81	10.5
Plant 2	19.15	10.82	7.25	8.27	20.0
Plant 3	13.85	10.40	6.42	7.23	30.0
Average	13.89	9.31	5.90	6.77	20.17
INTERNODE 2					
Plant 1	10.48	6.44	4.92	4.72	9.0
Plant 2	18.08	7.09	7.19	6.80	15.5
Plant 3	12.06	8.63	5.99	5.99	25.0
Average	13.54	7.38	6.06	5.84	16.5
INTERNODE 3					
Plant 1	11.01	5.29	4.42	4.79	13.5
Plant 2	19.95	4.66	6.32	6.52	16.5
Plant 3	18.17	8.26	6.51	6.65	20.0
Average	16.38	6.07	5.75	5.99	16.67
INTERNODE 4					
Plant 1	16.32	3.76	4.74	5.61	19.0
Plant 2	21.40	1.01	5.30	6.89	15.0
Plant 3	21.20	3.68	6.18	6.09	15.0
Average	19.64	2.81	5.41	6.20	16.33
INTERNODE 5					
Plant 1	16.64	1.86	4.46	5.23	10.0
Plant 2	18.19	0.00	3.90	5.54	11.0
Plant 3	24.32	3.11	6.52	6.69	10.0
Average	19.72	1.66	4.96	5.82	10.33
INTERNODE 6					
Plant 1	14.10	0.62	3.15	4.70	7.5
Plant 2	11.60	0.00	2.57	4.26	6.0
Plant 3	29.28	0.00	6.31	7.36	10.0
Average	18.33	0.20	4.01	5.44	7.83
INTERNODE 7					
Plant 1	11.97	0.00	2.65	3.93	6.0
Plant 2	9.25	0.00	2.17	4.69	3.0
Plant 3	24.10	0.00	5.24	6.46	10.0
Average	15.11	0.00	3.35	5.02	6.33

[1] Internode 1 is basalmost.

TABLE 4.—TISSUE AREAS AND LENGTHS IN THE SEVEN BASAL INTERNODES OF THE SERIES MINUS PHOSPHORUS[1]

	AREA OF: (Areas in mm²)				LENGTH OF INTERNODE
	Pith	Xylem	Phloem	Cortex	
					mm.
INTERNODE 1					
Plant 1	14.80	6.12	4.79	4.70	16.5
Plant 2	17.54	19.10	8.73	7.52	15.0
Plant 3	20.00	10.70	7.75	7.60	15.0
Average	17.45	11.97	7.09	5.61	15.5
INTERNODE 2					
Plant 1	15.29	6.92	4.76	5.24	15.0
Plant 2	19.68	16.70	6.87	7.13	18.0
Plant 3	22.68	10.44	7.22	7.05	16.0
Average	19.22	11.35	6.28	6.48	16.33
INTERNODE 3					
Plant 1	18.66	5.55	4.20	4.93	16.0
Plant 2	23.78	13.53	7.43	6.99	24.0
Plant 3	26.17	7.44	5.97	6.73	20.0
Average	22.87	8.84	5.87	6.22	20.0
INTERNODE 4					
Plant 1	22.09	4.70	4.45	5.40	17.0
Plant 2	24.50	8.21	5.85	5.81	22.5
Plant 3	27.96	5.83	6.72	5.78	23.0
Average	24.85	6.25	5.67	5.66	20.83
INTERNODE 5					
Plant 1	20.55	2.55	3.41	5.85	17.0
Plant 2	27.20	8.74	4.99	6.17	18.5
Plant 3	30.80	3.97	5.09	7.61	27.5
Average	26.18	5.09	4.50	6.54	21.0
INTERNODE 6					
Plant 1	21.47	2.18	3.00	5.28	17.0
Plant 2	28.53	6.13	4.49	5.59	21.0
Plant 3	27.87	2.63	3.77	6.42	28.0
Average	25.96	3.65	3.75	5.76	22.0
INTERNODE 7					
Plant 1	19.15	1.42	2.08	5.00	15.0
Plant 2	29.40	4.12	4.25	5.53	20.0
Plant 3	24.37	1.90	3.34	5.36	25.0
Average	24.31	2.48	3.22	5.30	20.0

[1] Internode 1 is basalmost.

tiated. The average volume of the pith cells in all four series is presented numerically in Table 7, and diagramatically in Figs. 6 to 9.

The average size of individual pith cells of the control plants tend to become increasingly larger in the first four internodes, then the size decreases slightly and tends to become more or less constant. In the plants lacking calcium the average cell volume is greatest in the first internode, slightly exceeding the control. These cells show a general trend to become more reduced in volume in each successive internode, and the average cell volume is much less than that of the controls in the upper internodes; only one-tenth as great in the seventh internode. In plants lacking phosphorus the average volume of the pith cells increases from slightly less than the control in the first internode to very slightly greater in the third internode, and then progressively decreases in the upper internodes. Plants lacking both calcium and phosphorus contain very large pith cells in the first internode, with a volume more than twice as great as the pith cells of the control. The average volume is approximately the same between this series in the second internode, rises above it in the third and fourth internodes, then shows a trend to decrease in the upper internodes.

Table 5.—Tissue Areas and Lengths in the Seven Basal Internodes of the Series Minus Calcium and Minus Phosphorus[1]

	Area of: (Areas in mm²)				Length of internode
	Pith	Xylem	Phloem	Cortex	
					mm.
Internode 1					
Plant 1	15.30	13.23	8.54	6.86	11.5
Plant 2	21.96	14.32	11.82	7.93	14.0
Plant 3	18.05	17.51	10.83	7.76	17.0
Average	18.44	15.02	10.40	7.52	14.16
Internode 2					
Plant 1	16.80	12.02	7.83	5.62	14.5
Plant 2	20.58	15.14	11.98	7.89	12.0
Plant 3	22.92	17.07	12.00	8.95	15.0
Average	20.10	14.74	10.60	7.49	13.83
Internode 3					
Plant 1	18.64	11.95	7.51	6.12	20.0
Plant 2	23.07	13.50	10.94	7.42	16.5
Plant 3	24.36	10.38	9.51	7.78	24.5
Average	22.02	11.94	9.32	7.17	20.33
Internode 4					
Plant 1	19·80	7·06	6·06	5·33	30·0
Plant 2	23·19	9·79	8·40	7·95	17·0
Plant 3	34.35	8.92	8.80	9.04	26.0
Average	25.78	8.59	7.75	7.44	24.33
Internode 5					
Plant 1	22.91	6.26	5.71	5.74	41.0
Plant 2	38.99	6.98	8.69	9.93	25.0
Plant 3	39.50	4.80	7.73	8.94	28.0
Average	33.80	6.01	7.38	8.20	31.33
Internode 6					
Plant 1	24.79	4.28	5.75	7.75	32.0
Plant 2	34.28	4.35	7.59	9.79	29.0
Plant 3	34.84	2.21	6.04	8.40	26.0
Average	31.31	3.61	6.46	8.65	29.0
Internode 7					
Plant 1	21.08	2.23	4.76	5.73	30.0
Plant 2	25.77	1.95	5.97	9.00	33.0
Plant 3	27.04	1.49	3.90	6.60	22.0
Average	24.63	1.89	4.88	7.11	25.0

[1] Internode 1 is basalmost.

From the data in Tables 6 and 7 it was possible to calculate the average number of pith cells per internode in each series of plants. Such cells are formed by divisions of the apical meristem and subsequent meristematic activity in the young internode during elongation and differentiation of the primary tissues. These figures are presented in Table 8, and illustrated diagrammatically in Figs. 10 to 13. The general trend for all four groups of plants is thus seen to be an increase of the number of cells in each successive internode.

For the control plants there is a positive correlation between the length of a particular internode and the number of cells in the pith, whereas in the three deficiency series of plants the correlation is negative; as the internodes become shorter the number of cells in the pith increases. Plants lacking phosphorus showed the greatest amount of activity of the apical meristem, exceeding the control plants; it was nearly as great in the plants grown without both calcium and phosphorus, again exceeding the number of cells in the pith of the control, with the exception of the sixth internode, in which the total number of pith cells fell below that of the controls. In the series minus calcium the actual number of pith cells per internode was smaller than the number in the control.

Histochemical Observations

Histochemical techniques make possible in most instances the exact location of a substance within a particular tissue, and often within the organelles of the cells themselves. Tests for a number of substances were carried out during the present study, and in those instances where the chemical composition was found to differ

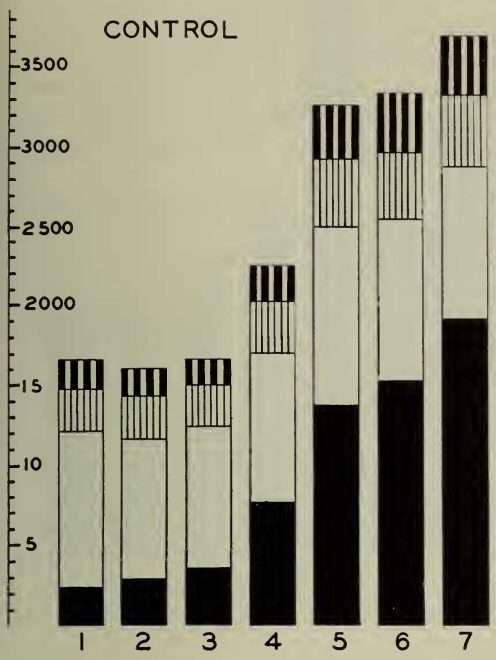

Fig 2

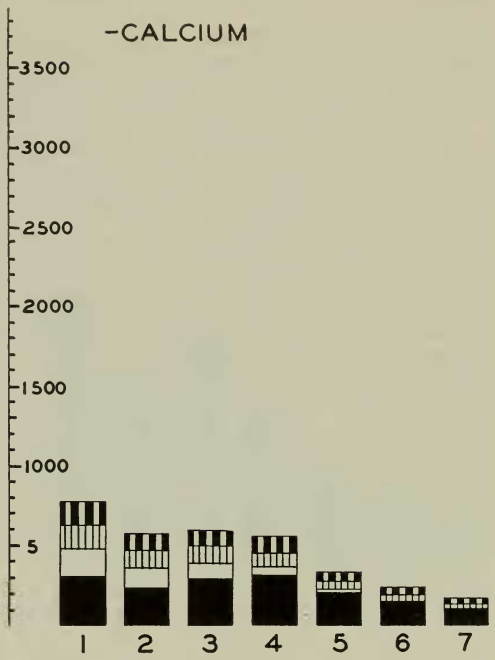

Fig 3

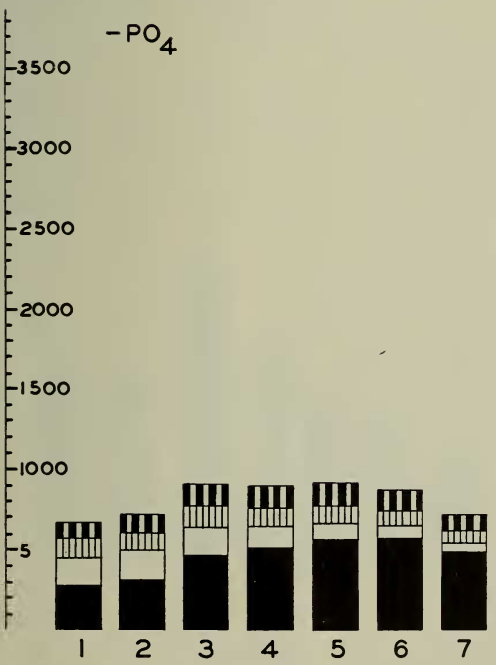

Fig 4

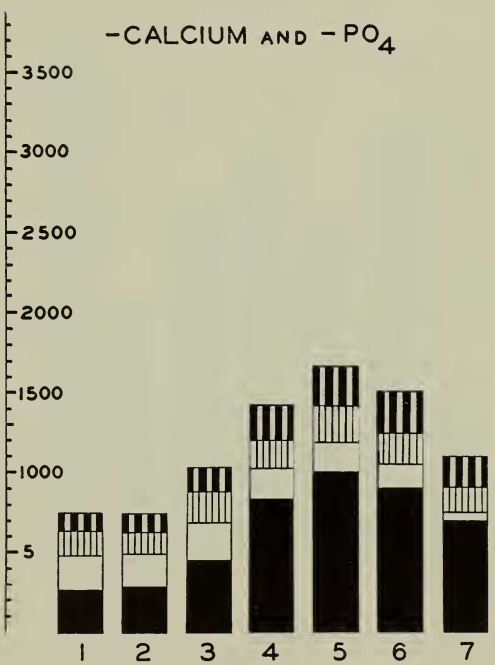

Fig 5

Figs. 2–5.—Comparative tissue volumes in the seven basal internodes of the four series of plants. Scale in cubic millimeters. ||| = cortex, ▥ = phloem, □ = xylem, ■ = pith. Note the absence of secondary xylem in internodes 6 and 7 in the series grown without calcium, and the greater volumes of tissue in the plants grown without both calcium and phosphorus than in the series lacking only one of these elements.

between the four series, a comparative quantitative evaluation was attempted. Such data are presented in detail in the following paragraphs.

Calcium was fairly abundant in all living cells in the axis of the control plants; it did not appear in association with the middle lamellae or cell walls, but was always seen in the protoplasts as small granular inclusions. In tissues such as the cambium the calcium granules were of very small size and finely dispersed throughout the cytoplast; in highly vacuolate tissue, such as the pith, they appeared as coarse inclusions. Some calcium was indicated in the lumena of vessels and tracheids in the xylem, suggesting continued uptake or translocation of these ions at the time of sampling. Although a reaction for calcium was obtained from all living cells the pith appeared to serve to some extent as a storage tissue for calcium, and the chlorophyll-bearing tissues, such as the cortex, were particularly rich in calcium, as was the epidermis. All seven basal internodes showed the same general distribution of this element, and in approximately the same quantity. Very young tissue taken from near the stem tip also showed the same distribution of calcium, and it was absent from the cell walls in this region also.

In the minus-calcium series, as would be anticipated, the total quantity of calcium in the tissues of the axis was far below that in the control plants. The quantity of recognizable calcium was relatively large, however, considering that it was obtainable only from the endosperm of the seed, and possibly to a limited extent from the tap water with which the seedlings were watered during germination. As in the controls it appeared as small granular inclusions in the cytoplasts of living cells, but unlike the control plants some of the calcium present was found in association with the pectic materials of the cell walls and middle lamellae. The conducting elements of the xylem were free from calcium. As in the control, the

TABLE 6.—COMPARATIVE TISSUE VOLUMES IN THE FOUR SERIES

(All figures given in mm³)

	CONTROL	MINUS Ca	MINUS PO_4	MINUS Ca and PO_4
INTERNODE 1				
Pith	242.5	296.6	269.2	263.4
Xylem	979.3	199.7	182.6	216.8
Phloem	268.2	126.6	108.8	149.3
Cortex	168.6	144.3	101.5	107.3
INTERNODE 2				
Pith	308.8	225.4	315.5	278.2
Xylem	888.2	127.9	190.5	204.0
Phloem	259.9	101.9	103.5	145.8
Cortex	146.0	99.3	106.6	103.5
INTERNODE 3				
Pith	360.8	280.4	464.3	450.1
Xylem	889.0	104.5	187.4	238.6
Phloem	253.4	98.0	121.7	187.8
Cortex	163.5	101.8	127.1	145.2
INTERNODE 4				
Pith	785.7	316.4	523.3	627.2
Xylem	935.8	47.2	133.0	203.4
Phloem	324.7	87.4	120.6	184.5
Cortex	211.9	100.5	118.5	176.7
INTERNODE 5				
Pith	1,387.7	203.3	566.5	1,000.7
Xylem	1,125.7	16.6	104.7	188.6
Phloem	419.3	50.9	96.8	222.6
Cortex	326.6	60.1	140.1	244.6
INTERNODE 6				
Pith	1,536.5	156.1	581.5	897.8
Xylem	1,033.7	1.6	79.8	158.3
Phloem	421.7	34.1	83.6	187.0
Cortex	341.3	44.8	128.9	250.2
INTERNODE 7				
Pith	1,925.4	113.6	494.6	692.6
Xylem	961.1	0.0	50.4	54.7
Phloem	457.5	24.9	66.5	141.9
Cortex	369.9	34.1	106.5	204.7

TABLE 7.—AVERAGE CELL SIZE IN THE PITH OF ALL FOUR SERIES

	RADIAL DIAM. (microns)	TANGENTIAL DIAM. (microns)	LENGTH (microns)	VOLUME (thousandths of a mm³)
INTERNODE 1				
Control	105	96	65	.52
−Ca	95	86	87	.56
$-PO_4$	106	93	55	.43
−Ca and $-PO_4$	116	101	119	1.10
INTERNODE 2				
Control	123	114	50	.55
−Ca	98	96	53	.39
$-PO_4$	110	106	45	.41
−Ca and $-PO_4$	97	96	72	.53
INTERNODE 3				
Control	116	117	57	.61
−Ca	110	102	55	.49
$-PO_4$	115	117	59	.62
−Ca and $-PO_4$	107	112	79	.74
INTERNODE 4				
Control	132	132	53	.73
−Ca	100	96	45	.34
$-PO_4$	105	109	49	.44
−Ca and $-PO_4$	121	115	76	.83
INTERNODE 5				
Control	126	127	95	1.19
−Ca	78	81	40	.20
$-PO_4$	92	92	54	.36
−Ca and $-PO_4$	112	104	78	.72
INTERNODE 6				
Control	126	122	79	.95
−Ca	79	80	28	.14
$-PO_4$	83	88	54	.31
−Ca and $-PO_4$	119	112	86	.90
INTERNODE 7				
Control	112	112	108	1.06
−Ca	69	70	27	.10
$-PO_4$	78	81	43	.21
−Ca and $-PO_4$	71	76	74	.31

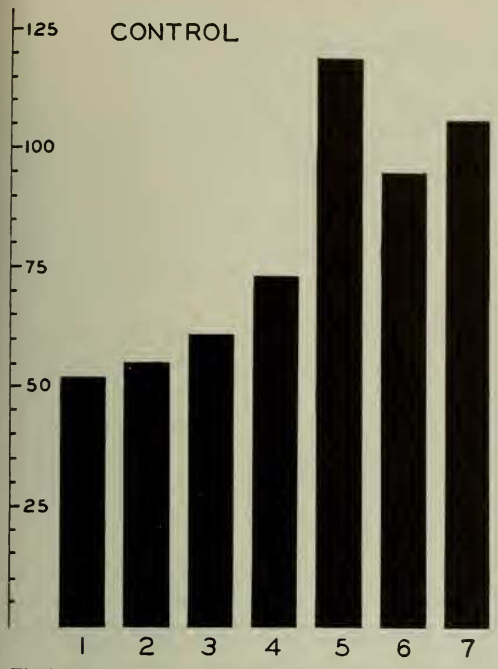

Fig 6

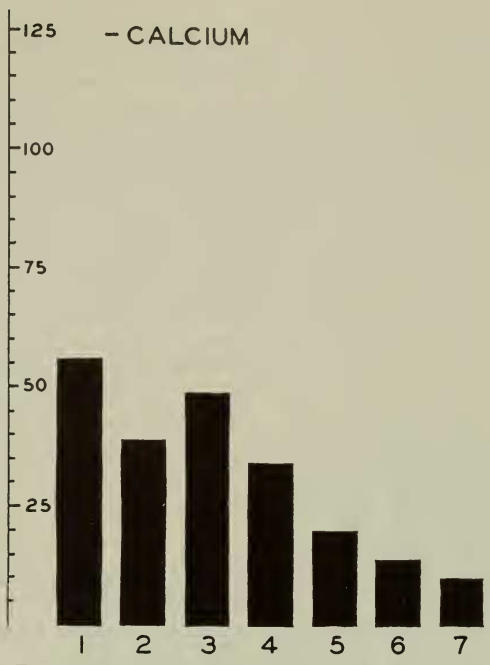

Fig 7

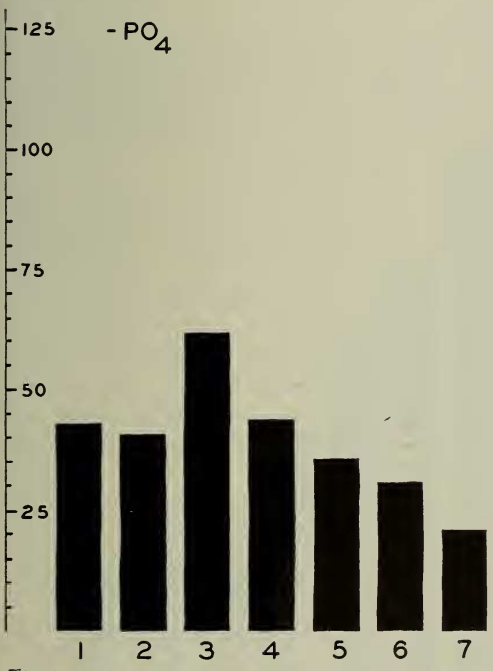

Fig 8

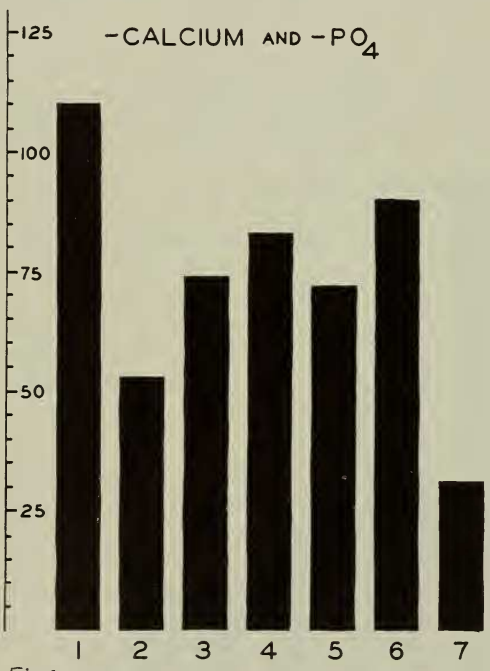

Fig 9

FIGS. 6–9.—Average cell size of the pith cells in the seven basal internodes of the four series of plants. One unit of the scale equals one ten-thousandth of a cubic millimeter. Note that in the control there is a gradual increase in size of the pith cells in internodes 1 to 5, and then the size tends to become more or less constant, whereas in the series minus calcium and the series minus phosphorus there is a general tendency for the size of the pith cells to become smaller and smaller in the upper internodes. It is seen that in the series lacking both calcium and phosphorus the pith cells in the first internode have volumes more than twice as great as the pith cells of the control; the average volume is approximately the same between this series and the control in the second internode, rises above it in the third and fourth internodes, then shows a trend to decrease in the upper internodes.

calcium present was most noticeable in young phloem tissue and in the chlorophyllous tissues of the stem, and in the epidermis. Sections from the upper internodes near the stem tip were also examined for traces of calcium, and the histochemical reaction indicated that the calcium available to these plants was rather evenly distributed throughout the plant body. The pith, while showing traces of calcium in the cell walls, had few or no cytoplasmic inclusions of calcium as were seen in the control plants. Occasional cells near the center of the pith which were dead were completely free of calcium, suggesting that it had been translocated to other regions.

The quantity and distribution of calcium in the minus-phosphorus series were similar to the controls, with abundant calcium in the cytoplasts of pith, cortex, and epidermis, and to a lesser extent in the cytoplasts of other tissues. Unlike the control plants, calcium was also associated with the cell wall substances of most cells in all stem tissues. The general distribution of calcium remained the same between internodes one and seven, but the quantity increased progressively upward. Sections from near the stem tip contained higher amounts of this element than did comparable regions in the control plants. As in the series grown without calcium, the occasional dead cells towards the center of the pith were completely free of calcium.

In the minus-calcium and minus-phosphorus series, the quantity and distribution of calcium closely resembled those in the series grown without calcium alone. Those traces of calcium present were also generally distributed throughout the axis in these plants. The same increase of the element in the cell walls and decrease in the cytoplasts as one moved upward in the axis was also noted.

Calcium oxalate occurred frequently in the pith of the control plants as large crystals, or druses, and was also frequent in the medullary sheath, older phloem, and inner cortex; and was occasionally seen in the hypodermis. In the young phloem calcium oxalate was abundant in the phloem rays across this tissue. There was a gradual decrease in the frequency of calcium oxalate druses from internode to internode upward in the stem, but considerable numbers were still present in the seventh internode.

In the basalmost internode of the series Minus Calcium, relatively large druses of calcium oxalate were encountered frequently in the pith, and a few were present in the medullary sheath. None were found outside the stele in the cortical tissues. The frequency of calcium oxalate druses was greatly reduced in the second internode, but those present were large and well-formed. Only a few small crystals of calcium oxalate occurred in the third internode, and none was found in the successive internodes.

In the minus-phosphorus plants calcium oxalate crystals were frequent in all seven basalmost internodes, in this respect resembling the control plants. They were located principally in the pith and medullary sheath. Unlike the control plants, only occasional cells containing calcium oxalate were found in the tissues to the outside of the cambium.

The minus-calcium and minus-phosphorus plants contained a much smaller quantity of calcium oxalate than any of the other series. A few cells containing small crystals of calcium oxalate were seen in the pith of the first internode only, and no other indications of this substance could be secured from any of the higher internodes.

Magnesium was scanty in the main axis of the control plants. Histochemical tests indicated a small amount in the pith of the three basalmost internodes; the upper internodes apparently contained too small a quantity of magnesium to give a positive reaction.

In the series minus calcium, magnesium was present in all tissues of the axis, and a progressive increase in quantity was noted from internode to internode upward. A large amount of magnesium was present in the upper internodes.

The plants grown minus phosphorus showed a trace of magnesium in the pith in all seven basal internodes, but none in any other tissue. In respect to magnesium this series was very similar to the controls.

Magnesium was rather liberally present in all

TABLE 8.—AVERAGE NUMBER OF PITH CELLS PER INTERNODE IN ALL FOUR SERIES

(Figures in thousands of cells)

	CONTROL	MINUS Ca	MINUS PO_4	MINUS Ca and PO_4
INTERNODE 1	466	530	626	239
INTERNODE 2	562	578	770	525
INTERNODE 3	592	572	749	608
INTERNODE 4	1,076	930	1,189	756
INTERNODE 5	1,167	1,016	1,574	1,390
INTERNODE 6	1,617	1,115	1,876	998
INTERNODE 7	1,816	1,135	2,357	2,234

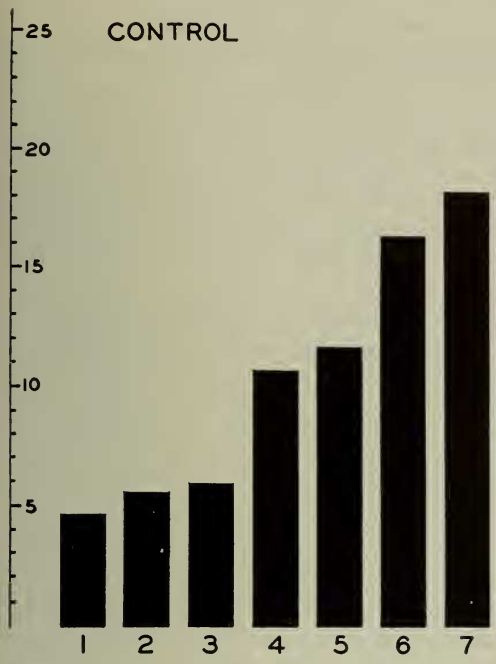

Fig 10

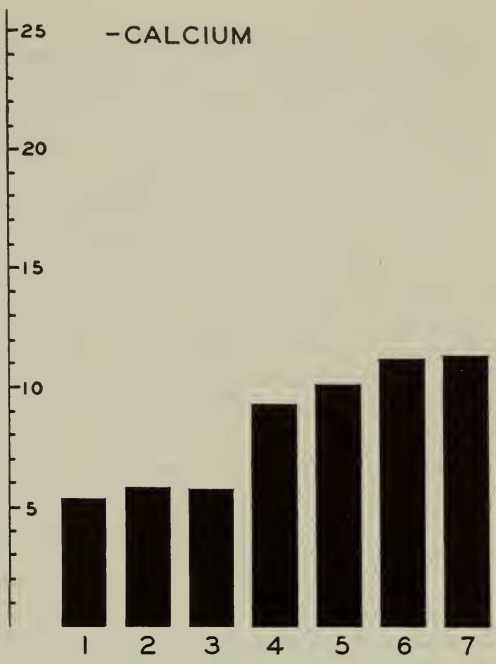

Fig 11

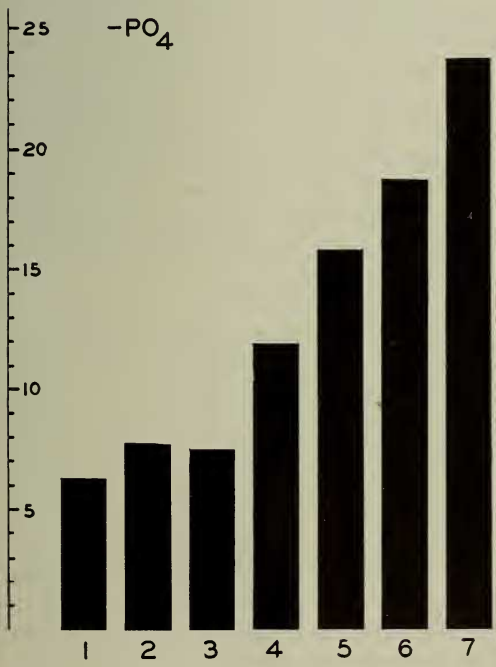

Fig 12

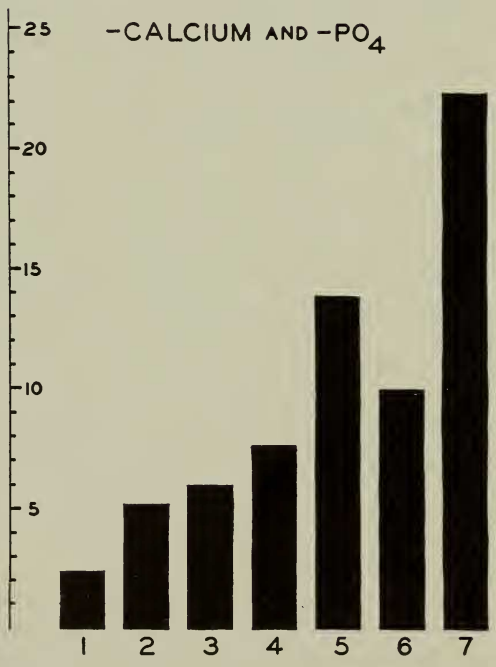

Fig 13

FIGS. 10–13.—Average number of pith cells per internode in the seven basal internodes of the four series of plants. Scale: 1 unit equals 100,000 cells.

the tissues of the axis in the series minus calcium and minus phosphorus. It seemed most abundant in parenchymatous tissues; a slightly lesser quantity was in the xylem. There was a greater amount of magnesium in the tissues of plants in this series than in any other.

Phosphates. No positive reaction for phosphates could be obtained in the seven basal internodes in all four series. Apparently at the time of sampling there was too small a quantity of phosphates in the lower axis to give a reaction. Positive tests for phosphates were secured from branches of the inflorescence and sections close to the apical meristem of the control plants and the plants grown without calcium; none could be detected from these structures in the two series not supplied with phosphates.

Nitrates and nitrites were present in fairly large quantity in all four series. The series grown without both calcium and phosphorus contained a slightly larger amount of these substances than did the three other series, but the plants in all four series contained what would seem to be adequate amounts.

Protein nitrogen was detectable in fairly heavy amounts in the controls and in the series minus calcium and minus phosphorus; the series minus calcium contained by far the greatest quantity. The plants minus phosphorus gave the lowest reaction for protein nitrogen, restricted to the xylem parenchyma, prosenchyma, and pith rays.

Sulfates and organic sulfur, when present in a tissue, did not seem to be evenly distributed throughout the tissue, but were restricted to certain cells, otherwise indistinguishable except for the concentration of these substances. No sulfates were detected in the series minus calcium nor in the series minus phosphorus. In the controls and the series deficient in both calcium and phosphorus a medium amount of sulfates were found in the pith of all seven basal internodes. Organic sulfur was often associated with tannin cells in the cortex, phloem, and epidermis.

Potassium was abundant in all tissues of the axis in all four series.

Fructose gave a trace reaction in the pith of the controls and the series minus calcium; none was present in the two series lacking phosphorus.

Glucose in very large quantities was observed in all tissues of the control plants, and even larger amounts in the series lacking phosphorus, and in that lacking both calcium and phosphorus. The series grown without calcium alone contained considerably less glucose, but it was still present in rather large quantity.

Amylodextrin was indicated only in those series which received calcium. It appeared in special scattered cells in the pith of the control, and in the cortex and epidermis as well in the series lacking phosphorus, but was never generally distributed throughout a particular tissue.

Starch was present in considerable quantity in all plants. The series minus calcium contained the largest amount, principally in the pith, while the series minus phosphorus contained less starch than the control. The smallest quantity was observed in the series lacking both calcium and phosphorus.

Fats and oils were indicated as small droplets or globules in the cytoplast. Such organelles were markedly associated with the chloroplasts in the green portions of the stem. In the control plants and those grown without calcium only, fats and oils were evenly distributed in all seven internodes studied.

Both series grown without phosphorus lacked the small globular cytoplasmic inclusions in the lower internodes, but the nuclei and nucleoli of almost all the cells of these internodes did stain bright red with Sudan IV, presumably from lipoidal materials. In the upper internodes in these series the number of globules of fats and oils in the cytoplast increases, so that in the uppermost internodes all living tissues contain at least traces of these substances. All series of plants had well-developed cuticles on the stems.

DISCUSSION

In addition to its function as support for the aerial organs and translocation between the various parts of the plant body, the stem is of necessity the structure through which takes place any coordination of development and function between the component parts of the plant. In particular, cambial activity and the development of secondary vascular tissues seem to be closely coordinated with the degree of necessity for conduction between and mechanical support of the lateral organs. Knight (1803) and Vöchting (1918) demonstrated that in dicotyledons the quantity of mechanical tissue in a stem was directly proportional to the mechanical stress imposed by the lateral organs; Kohl (1886) and Jost (1907) established that the size and number

of foliage leaves, or the rate of transpiration, or both, directly influenced the development of the conducting tissues in the axis.

Although it is possible to conceive all differences in axial structure between the four series of plants grown in this experiment to have been ultimately brought about by differences in mineral nutrition, the basis for the actual amount of cambial activity and development of secondary vascular structure could in part depend on the functions the axis was called on to perform for the lateral organs. In the deficiency series of plants, where reduced leaf area and loss of foliage at the time of flowering occurred, a corresponding reduction in mechanical and water-conducting tissues was noted; its reduction was more or less proportional to the loss of leaf area. The quantity of phloem, however, was seen to remain relatively large. Similar observations in castor bean were made by Penfound (1932), who concluded that conditions favoring rapid water adsorption and rapid water loss also give the greatest development of vascular tissue.

There is little evidence that cell divisions and subsequent vacuolation and elongation near the stem apex are as directly influenced by the size and functions of the leaves. Indirect evidence tends to show the opposite to be the case, with the older foliage sacrificed to the development of fruits and to the continued growth of the apical regions.

In the castor bean, plants grown without calcium, with or without phosphorus, showed a decrease in the traces of calcium in the protoplasts of the upper internodes, accompanied by a decrease of calcium oxalate and a marked increase in magnesium. With these changes there was a reduction in length of the internodes, and an increase in the number of cells per internode, but in the series lacking calcium alone the increase in cell number was below that in comparable internodes of the control plants. Similar effects of calcium deficiency have been noted by previous investigators. Loew (1892) pointed out the increased concentration of magnesium in wheat plants deficient in calcium, and subsequently concluded (1903) that the increased concentration of magnesium in the cells exerted a toxic effect on the tissues. This view was supported by Reed (1907), who stated that in addition to calcium appearing necessary for activity and growth of chlorophyll-bearing tissue, one of its most important functions seemed to be that of overcoming the bad effects of magnesium. It has also been suggested (Groom, 1896) that a deficiency in calcium leads to an accumulation of oxalic acid, a by-product of protein synthesis, with subsequent toxic effects which are normally overcome by calcium, which precipitates this waste by forming the almost insoluble salt, calcium oxalate. Inhibition of stem elongation in calcium-deficient garden peas, *Pisum sativum* L., was reported by Day (1928, 1929), in which she noted that the anatomical structure of the stem and root remained constant (as compared to control plants) and that the differences were principally the degree to which the stems had elongated. Davidson and Blake (1936) made a similar observation on calcium-deficient peach trees, and reported the stem growth restricted in length but not greatly restricted in diameter. Fewer cells of smaller size than normal were described from the stem tips of loblolly pine, *Pinus taeda* L., by Davis (1949). The inhibiting of cell division and elongation, as observed in the instances cited above and in castor bean, might be supposed to be caused by (1) the direct necessity for calcium as a protoplasmic component, together with the toxic effects of an accumulation of oxalic acid, or (2) the excess accumulation of other minerals, such as magnesium, which are thought toxic in large quantity or when not in antagonism with calcium, or (3) the action of other substances or minerals normally present, whose action on cellular activity is masked by the presence of calcium.

Plants in the series lacking phosphorus showed reduction in internodal length and cell size in the upper internodes, with a much greater increase in the number of cells per internode than in the control plants. These differences were associated with a reduced amount of fats and oils in the protoplast, and a smaller amount of protein nitrogen, with an increased quantity of calcium. Similar observations were described for phosphorus-deficient tomatoes by Eckerson (1931). Disintegration of central pith cells as described in tomato by Lyon and Garcia (1944) was not observed, but many of the central pith cells were dead in the internodes studied. Reduced cell size, as seen in the upper internodes of castor bean, has been reported for phosphorus-deficient tomatoes by Watts (1938); apparently there are no reports of increased meristematic activity in the apical tissues associated with this deficiency. The differences in the upper internodes between

this and the control series of plants could be interpreted to imply (1) that phosphorus, in addition to its other metabolic roles, may act as an inhibitor or regulator of cell division, and its deficiency removes a check on this process, or (2) abundant calcium is a stimulator of cell division, or (3) the action of calcium or of other substances normally present is changed by the absence of phosphorus.

The series lacking both calcium and phosphorus shows a curious makeup of similarities and differences between the series deficient in only one of these elements and the control series. Like the series deficient in calcium alone, they show a decrease of calcium in the protoplasts of the cells in the upper internodes, a decrease in the amounts of calcium oxalate, and a marked increase in the amounts of magnesium in the tissues. Unlike the series deficient in calcium alone, they contain larger quantities of sulfates, and smaller amounts of fats, oils, and starch.

As compared with the series deficient in phosphorus, these plants were similar in their reduced amounts of fats and oils, but unlike in the smaller amounts of starch, reduced calcium, lack of calcium oxalate, and high magnesium content. They showed a greater number of cells in most of the upper internodes than did the controls, but not so large a number as in the series lacking phosphorus alone.

From comparisons between this and the other series, some indications of calcium and phosphorus activity as related to cell divisions are suggested:

Calcium has long been thought to play an important part in the process of cell division, not only in the mitotic process, but also in the formation of the cell plate and middle lamella. Numerous experiments in which plants were grown on a complete nutrient solution in which sodium, barium, strontium, or magnesium were substituted for calcium have yielded similar results: erratic cell divisions in the meristems which became fewer and finally ceased, and vacuolation of the cells of the root meristems associated with damage and death to these tissues, and in cases of extreme calcium deficiency followed by death to the plant as a whole (Bruch, 1902; True, 1922; Mevius, 1927; Harris, 1928; Sorokin and Sommer, 1929; Albrecht and Davis, 1929; Nightingale, 1937; Tucker and Burkholder, 1941; and Davis, 1949). Bamford (1931) performed similar experiments on the roots of wheat and corn seedlings; the root tips were permanently injured after one day in solutions lacking calcium, whereas the plants throve in the same solution were calcium present.

Bamford's observations went yet further; no significant root injury was demonstrated after submergence for seven days in distilled water, which of course supplied no calcium. This evidence indicated that the rapid root injury observed when roots were placed in a nutrient solution from which calcium had been omitted could not have been due to the failure of this solution to supply calcium to the roots. He concluded that injury must have resulted from the unbalanced condition of the cultural solution, the components of which were toxic when not antagonized by calcium.

The roots of castor bean showed such injury when grown without calcium, whereas similar damage was not apparent to the roots of plants lacking both calcium and phosphorus. Similarly, cell divisions were inhibited in the upper internodes of the plants lacking calcium alone, whereas for the most part there were a greater number of cells per internode in the plants lacking either phosphorus or both calcium and phosphorus than in the control plants. Although necessary as a protoplasmic constituent, it would thus appear that phosphorus, when not associated with calcium, has a toxic effect on root meristems and to some extent on apical meristems, and that there are reciprocal effects between ions of calcium and phosphorus which reduce the toxic effects of the latter. The fact that the plants lacking only phosphorus died as a result of fruiting, while those lacking both calcium and phosphorus did not, would suggest that calcium, in the absence of phosphorus, may have deleterious metabolic effects normally masked by phosphorus.

The toxicity of magnesium in the plant body would not appear to be as great as previously supposed; comparisons between the series lacking phosphorus, with a low magnesium–high calcium content, and the series lacking both calcium and phosphorus, in which the reverse situation of a high magnesium–low calcium content was found, showed both to have a greater number of cells per internode in the upper internodes than did the controls. Extensive root damage in the series lacking calcium, and no root damage in the series lacking both calcium and phosphorus, both of which received large amounts of magnesium, would seem to indicate that the magnesium was not responsible for the damage seen in the one series.

Both calcium and phosphorus would appear to be necessary for cell elongation, as elongation was eventually reduced in plants lacking one or both of these elements.

SUMMARY

The basic anatomical pattern and arrangement of tissues in the axis of castor bean is conservative; deficiencies of calcium and phosphorus do not affect the basic anatomical pattern, but are reflected in the quantities of tissue produced. A deficiency of either calcium or phosphorus alone has greater adverse effects to the plant than when both elements are lacking.

The effects of calcium and phosphorus deficiency on the growth and development of castor bean are much more profound when the plant is in the flowering and fruiting phases of development than when in vegetative growth.

The activity of the vascular cambium, and differentiation of secondary conducting and mechanical tissues in the axis is restricted in both deficiencies. This reduction in secondary tissues parallels loss of foliage area, and is probably in part a response to such loss, rather than a response solely to differences in chemical composition of the axis.

The number of pith cells per internode provide an index of previous primary (apical) meristematic activity. Calcium deficiency results in a smaller number of cells per internode than in control plants, phosphorus deficiency in a much larger number of cells per internode than in the controls, and a deficiency of both calcium and phosphorus produced a greater number of cells per internode than in the controls, but a smaller number than in plants lacking phosphorus alone.

The phosphate ion appears to be toxic to meristematic cells when not associated with calcium, there being a reciprocal effect between ions of the two elements which reduces the toxicity of the latter. Calcium appears to have a stimulatory effect on cell division which is somewhat curtailed when associated with phosphorus. Both calcium and phosphorus appear necessary for cell elongation.

LITERATURE CITED

Albrecht, W. A., and Davis, F. L. *Physiological importance of calcium in legume innoculation.* Bot. Gaz. **88:** 310–321. 1929.

Bamford, R. *Changes in root tips of wheat and corn grown in nutrient solutions deficient in calcium.* Bull. Torrey Bot. Club. **58:** 149–178. 1931.

Bruch, F. *Physiological importance of calcium in plants.* Landw. Jahrb. **30:** Erg. Bd. **3:** 127–143. 1902.

Chamberlain, C. J. *Methods in plant histology,* ed. 5. Chicago, 1932.

Davidson, O. W., and Blake, M. A. *Responses of young peach trees to nutrient deficiencies.* Proc. Amer. Soc. Hort. Sci. **33:** 247–248. 1936.

Davis, D. E. *Some effects of calcium deficiency on the anatomy of* Pinus taeda. Amer. Journ. Bot. **36:** 276–282. 1949.

Day, D. *Some effects of* Pisum sativum *of a lack of calcium in the nutrient solution.* Science **68:** 426–427. 1928.

———. *Some effect of calcium deficiency on* Pisum sativum. Plant Physiol. **4:** 493–506. 1929.

Eckerson, S. H. *Influence of phosphorus deficiency on metabolism of the tomato.* Contrib. Boyce Thompson Inst. **3:** 197–217. 1931.

Foster, A. S. *Practical plant anatomy,* ed. 2, New York, 1949.

Groom, P. *On the relation between calcium and the transportation of carbohydrates in plants.* Ann. Bot. **10:** 91–96. 1896.

Harris, J. A. *Studies of the elements required in only small quantities for the development of the green plant and miscellaneous investigations.* Activities of the Department of Botany, University of Minnesota, for 1927. 1928.

Johansen, D. A. *Plant microtechnique.* New York, 1940.

Jost, L. *Lectures on plant physiology.* Berlin, 1907.

Knight, *in* Haberlandt, G. *Physiological plant anatomy*: 194–195. (Transl. 4th German ed. by M. Drummond.) London, 1928.

Kohl. *Die Transpiration der Pflanzen:* 90 et seq. Braunschweig, 1886.

Loew, O. *Über die physiologischen Funktionen der Ca- und Mg-Salze im Pflanzenorganismus.* Flora **75:** 368. 1892.

———. *Unter welchen Bedigugen wirken Mg-Salze shadlich auf Pflanzen?* Flora **92:** 489. 1903.

Lyon, C. B., and Garcia, C. R. *Anatomical responses to tomato stems to variations in the macronutrient anion supply.* Bot. Gaz. **105:** 394–405. 1944.

Mevius, W. *Kalzium-Ion und Wurzelwachstum.* Jahrb. für wiss. Bot. **66:** 183–253. 1927.

Nightingale, G. T. *Potassium and calcium in relation to nitrogen metabolism.* Bot. Gaz. **98:** 725–734. 1937.

Penfound, W. T. *The anatomy of the castor bean as conditioned by light intensity and soil moisture.* Amer. Journ. Bot. **19:** 538–546. 1932.

Reed, H. S. *The value of certain nutritive elements to the plant cell.* Ann. Bot. **21:** 501–543. 1907.

Sorokin, H., and Sommer, A. L. *Changes in the cells and tissues of root tips induced by the ab-*

sence of calcium. Amer. Journ. Bot. **16**: 23–39. 1929.

TRUE, R. H. *The significance of calcium for higher green plants*. Science **55**: 1–6. 1922.

TUCKER, C. M., and BURKHOLDER, P. R. *Calcium deficiency as a factor in abnormal rooting of philodendron cuttings*. Phytopath. **31**: 844–848. 1941.

VÖCHTING, H. VON. *Untersuchen zur experimentellen Anatomie und Pathologie der Pflanzenkorpers* **2**: Tübingen, 1918.

WATTS, V. M. *Anatomical symptoms of nitrogen, phosphorus, and potassium deficiencies in seedling hypocotyls of tomato* Lycopersicum esculentum *Mill.*). Bull. Arkansas Agr. Exp. Stat. 366. 1938.

MYCOLOGY.—*A nematode-capturing fungus with clamp-connections and curved conidia.* CHARLES DRECHSLER, United States Department of Agriculture, Plant Industry Station, Beltsville, Md.

In earlier papers (Drechsler, 1941, 1943, 1946, 1949) I described as new species six nematode-destroying fungi that may with some confidence be reckoned among the Basidiomycetes, for although they have not been found producing basidia and basidiospores their hyphae are unmistakably furnished with clamp-connections. Four of these fungi, namely *Nematoctonus tylosporus*, *N. leiosporus*, *N. pachysporus*, and *N. leptosporus*, always attack eelworms in the usual manner of parasites: their conidia, after becoming externally affixed to the animal by means of an adhesive secretion, will push through the integument a narrow germ tube which on reaching the fleshy interior widens out, elongates, and ramifies to form an assimilative mycelium extending lengthwise from head to tail. The two other fungi, *N. haptocladus* and *N. concurrens*, likewise often attack by intruding a germ tube from an adhering conidium, but in addition they employ adhesive organs of mycelial origin to capture motile eelworms; each captive being subsequently invaded and expropriated of all its digestible substance. A clamp-bearing fungus similarly given to capture of nematodes but differing markedly in its strongly curved conidia from both *N. haptocladus* and *N. concurrens*, as well as from the other 4 named species of *Nematoctonus*, was mentioned (Drechsler, 1941, p. 780) as occurring in Hawaii, though the material available at the time was too poor to justify a full description under a separate binomial. More recently a nematode-capturing fungus with clamp-connections and strongly curved conidia developed abundantly in several maize-meal agar plate cultures which after being over-grown by *Pythium debaryanum* Hesse had been further planted with small quantities of decaying vegetable detritus collected on December 20, 1952, in an open field in southern Louisiana. How this fungus is related to the Hawaiian form remains uncertain. In any case it seems unquestionably distinct from the six species of *Nematoctonus* to which names have been given, and accordingly merits recognition as an additional member of the genus. A specific epithet compounded of two words (καμπυλος and σπορα) meaning "bent" and "seed," respectively, may serve helpfully in recalling one of its most conspicuous diagnostic features.

Nematoctonus campylosporus sp. nov. Hyphae assumentes incoloratae, plus minusve ramosae, plerumque circa 2μ crassae, intra vermiculum nematoideum crescentes, post mortem animalis hyphas procumbentes (vel rarius ascendentes) extra emittentes; his hyphis procumbentibus incoloratis, aliquid ramosis, ad modum Hymenomycetum septato-nodosis, hic illic (praecipue in nodis) sterigmata ferentibus, saepe 25–200μ longis, ex magna parte in cellulis filiformibus 10–50μ longis et 1.7–2.5μ crassis constantibus, sed cellula paenultima in postica ejus parte saepius 2–3.5μ crassa in antica ejus parte vulgo usque 1.5μ attenuata et abrupte in aerem flexa itaque fronte in modo columellae ascendente; columella circa 5μ alta, 1.6–2μ crassa, cellulam ultimam in aere sustentans; cellula ultima saepius 3.5–5μ longa, 1.6–2μ crassa, medio aliquid constricta, primo nuda sed mox pila glutinis circumdata, denique saepe ad vermiculum nematoideum inhaerente, animal ita capiente, cuticulam ejus perforante, hyphas assumentes intrudente; sterigmatibus 2–5μ altis, sursum attenuatis, apice circa 0.5μ crassis, conidia singula ferentibus; conidiis incoloratis, allantoideis, plerumque valde curvis, basi atque apice late rotundatis, vulgo 10–13μ longis, 2.5–4μ crassis.